Fibrinolysis and platelet dysfunction during cardiopulmonary bypass

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SUMMARY AND CONCLUSIONS

Although nowadays cardiopulmonary bypass (CPB) can be considered safe, the inflammatory response and bleeding diathesis are still major drawbacks of CPB. In concert with the latter, this thesis is concerned with the affected haemostasis during CPB. Bleeding during CPB originates primarily from the surgical field by oozing from the capillaries. Although many causes are held responsible to some degree, most researchers agree that platelet dysfunction during CPB most likely is the main cause for the observed bleeding diathesis. The bleeding causes in many cases demand for donor blood, emphasising the need for preservation of haemostasis. Moreover, application of arterial instead of venous grafts for coronary artery bypass grafting and increasing usage of aspirin by cardiac patients amplify blood loss and increase the consecutive demand for homologous blood products after cardiac surgery.

This thesis, therefore, is focused especially on factors and circumstances during CPB which interfere with an adequate platelet function and, consequently, affect haemostasis.

Based on literature and observations in previous studies reviewed in **Chapter 2**, reversible impairment of platelet function during CPB was hypothesised. This dysfunction is characterised by a temporary loss of glycoprotein Ib receptors (GPIb), the binding site for von Willebrand factor and essential to the first phase of haemostasis. The **aim of this thesis** in **Chapter 1**, therefore, was to demonstrate a general mechanism responsible for the observed bleeding diathesis and platelet dysfunction during CPB, and to develop techniques to decrease the bleeding diathesis. All this was handled in several consecutive studies.

In **Chapter 3** the factors and activation patterns in blood collected during CPB, which are suspected to be related with the observed platelet dysfunction, were described. By analysis of samples from the circulating blood and from wound blood collected in the pericardial cavity during the operation, highly activated clotting and fibrinolysis in the wound blood compared to the circulating blood was demonstrated. Especially thrombin/antithrombin III complex (TAT), soluble fibrin and fibrinolytic degradation products (FDP) were much higher in pericardial blood. Once the pericardial blood was returned to the systemic circulation, TAT and FDP concentrations increased significantly also in the systemic blood. The effects of pericardial tissue to activation of clotting and fibrinolysis was also demonstrated in vitro. Inhibition of thrombin activity (clotting) by the introduction of hirudin during these tests, also decreased the activation of fibrinolysis, demonstrating that fibrin products produced by activation of the clotting system initiated fibrinolysis. The results of the study strongly suggest that tissue dependent activation of clotting by the pericardial tissue

highly contributes to the systemic activation of fibrinolysis, thus affecting haemostasis during CPB.

For obtaining more defined data on wound blood and its possible effects on circulating blood after retransfusion, the effect of retransfusing postoperative shed blood was determined. In **Chapter 4**, significant correlations between concentrations of fibrinogen degradation products and soluble fibrin in shed blood and higher postoperative blood loss were observed. Furthermore, retransfusion of shed blood significantly increased the total postoperative blood loss by 43 % (925 vs. 1320 ml). Based on these clinical observations, an increased bleeding tendency related to renewed fibrinolysis was hypothesised.

In the second part of this chapter concentrations t-PA and soluble fibrin similar to those observed during CPB were introduced to platelet rich plasma. The effect of this treatment on platelet function was determined by a platelet agglutination test dependent on von Willebrand factor (vWF) and GPIb receptors (ristocetin agglutination). T-PA and soluble fibrin together induced severe platelet damage, resulting in a decreased ristocetin agglutination response. Therefore, a plasmin mediated platelet dysfunction during CPB was proposed, dependent upon factors such as fibrin monomers and t-PA.

For assessment of plasmin generation in the presence of platelets, in **Chapter 5** a spectrophotometric method was customised for monitoring plasmin generation in mixtures which contain both plasminogen activators and platelets. This kinetic model allowed assessment of synergistic effects of platelets and fibrin monomers on plasminogen activation. By kinetic analysis of the turnover of a plasmin specific chromogenic substrate, a value for plasmin generation was established. Gelfitered platelets alone, or in the presence of fibrin monomers, mediated no plasminogen activation. However, increasing activation of plasminogen by increasing amounts of platelets was found in the presence of t-PA and fibrin monomers. The obtained data suggest that platelets offer an appropriate surface for t-PA/fibrin monomers to activate plasminogen.

The method was applied in **Chapter 6**, in which plasminogen activation and receptor damage of platelets by the combined treatment of t-PA and fibrin monomers were measured and correlated. Also, the question was addressed whether this platelet dysfunction in vitro could be prevented pharmacologically, as suggested by the blood saving effects of fibrinolysis inhibitors during several clinical interventions. In this study the binding of soluble fibrin to the platelet surface was demonstrated. Platelets pretreated with fibrin monomers or platelets in a mixture with fibrin monomers, t-PA and plasminogen caused a significantly increased plasmin generation, dependent on t-PA, fibrin monomers and platelets in a dose dependent fashion. The plasmin generation resulted in a downregulation of platelet membrane GPIb/IX glycoprotein

complexes. Since high concentrations of fibrin monomers and elevated levels of t-PA were observed during CPB, plasmin activity directed towards membrane glycoproteins essential for an adequate haemostasis was anticipated. Especially the downregulation of GPIb/IX complexes would affect haemostasis and increase bleeding during and after CPB.

Finally, it was demonstrated that inhibitors of fibrinolysis, such as α_2-antiplasmin, tranexamic acid and aprotinin, can inhibit any generated plasmin activity in the fluid phase. Aprotinin also prevented downregulation of platelet GPIb/IX complexes, but α_2-antiplasmin and tranexamic acid could not. This might explain the success of aprotinin in preserving platelet function and preventing bleeding after CPB.

The developed hypothesis that wound blood from the thoracic cavity induces fibrinolysis and consequently platelet dysfunction during CPB, was challenged by changing the operation protocol in a controlled prospective clinical study, as presented in **Chapter 7**. In this study thoracic wound blood was collected separately. The wound blood was then retransfused to the patient at the end of operation, or was retained. The immediate and significant increase of circulating concentrations TAT, t-PA, FbDP and free plasma haemoglobin after retransfusion of wound blood demonstrated the retransfusion effect. Moreover, the increased concentrations of TAT and FbDP indicated renewed systemic clotting and fibrinolysis as a direct result of the retransfusion of wound blood. Concentrations of all indicators mentioned remained significantly lower in the retainment group.

The clinical data showed that retainment of thoracic wound blood resulted in significantly decreased postoperative blood loss (822 ml in the retransfusion group vs. 611 ml in the retainment group, $p<0.05$) and similar or even reduced consumption of blood products such as red blood cell concentrates and single donor plasma. It was concluded that retransfusion of highly activated wound blood during CPB affects haemostasis and enhances wound bleeding.

In conclusion this thesis demonstrates that the observed concentrations of clotting and fibrinolytic factors, such as soluble fibrin and t-PA, can induce fibrinolysis directed especially towards platelets. This mechanism is mediated by binding of fibrin monomers, t-PA and plasminogen to the platelet surface. The consequently generated plasmin will affect platelet glycoprotein Ib/IX expression and cause a transient platelet dysfunction. Retransfused wound blood is therefore identified as the main cause of impairment of haemostasis.

One of the implications of the findings in the thesis concerns biocompatibility assessment of the extracorporeal circuits, and is described in **Chapter 8**. Biocompatibility assessment and related haematologic effects of extracorporeal

circuits during cardiopulmonary bypass will be greatly influenced when other substantial blood activating mechanisms are manifest. The large changes in circulating concentrations of activated products, caused by the retransfusion of thoracic wound blood, will obscure effects induced by the extracorporeal circuit. In this clinical study it was demonstrated that some variables are unfit for characterisation of extracorporeal circuit biocompatibility. Moreover, assessment of extracorporeal circuit biocompatibility is only feasible when thoracic wound blood is retained, or washed before retransfusion.

A summary and general conclusions of the thesis are presented in **Chapter 9** (English) and **Chapter 10** (Dutch).

Some general background on the components and mechanism of haemostasis is provided in **Addendum I** of this thesis. A model for the mechanism of aprotinin (Trasylol®) related preservation of peri- and postoperative haemostasis during CPB, in line with the findings of this thesis, is proposed in **Addendum II.**